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Assessing amyloid- β , tau, and glial features in Lothian Birth Cohort 1936 participants post-mortem

Makis Tzioras, Jacqueline Easter, Sarah Harris, Chris-Anne McKenzie, Colin Smith, Ian Deary, Christopher Henstridge, Tara L Spires-Jones

Centre for Cognitive and Neural Systems, The University of Edinburgh; Centre for Cognitive Ageing and Cognitive Epidemiology, Medical Genetics Section, University of Edinburgh Centre for Genomic and Experimental Medicine and MRC Institute of Genetics and Molecular Medicine, The University of Edinburgh; Centre for Clinical Brain Sciences, The University of Edinburgh; Centre for Clinical Brain Sciences, Centre for Comparative Pathology, The University of Edinburgh; Centre for Cognitive Ageing and Cognitive Epidemiology, Department of Psychology, The University of Edinburgh; UK Dementia Research Institute, Centre for Discovery Brain Sciences, Edinburgh Neuroscience, Centre for Dementia Prevention, Euan MacDonald Centre for Motorneurone Disease Research, The University of Edinburgh

✉ **Correspondence**
tara.spire-jones@ed.ac.uk

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Abstract

The decline in cognitive function is one of the most feared aspects of ageing. We are yet to fully understand why some people age with relatively intact cognition, while others experience a subtle cognitive decline or even dementia. The Lothian Birth Cohort 1936 (LBC1936) was established to investigate lifetime cognitive changes, with data collected at 11 years of age and again at 70 years old, onwards. The individuals have been extensively characterised in terms of genetics, cognitive function, and biomedical, psychological, and lifestyle factors. This pilot study characterises and quantifies morphological and pathological features of the first nine donated brains from this cohort. Specifically, we have analysed amyloid-beta ($A\beta$), phosphorylated tau, microglia and astrocyte levels in five brain regions from nine non-demented LBC1936 participants' post-mortem brain tissue to determine how these factors vary between brain regions. Amyloid- β ($A\beta$) and phosphorylated tau tangles are hallmarks of Alzheimer's disease, the most prevalent form of dementia, although these have also been described in the brains of some non-demented aged individuals. In both ageing and dementia, immune-related changes are common, including microglia and astrocyte dysfunction. We found that tau tangles and glial cell coverage were highest in the hippocampus, in contrast to $A\beta$ which was more abundant in the neocortex. We anticipate that this cohort will provide invaluable information about brain changes during normal ageing, and act as an age-matched control group for studies investigating neurodegenerative disorders with significant cognitive impairment, such as Alzheimer's disease.

Introduction

A common and devastating aspect of growing older is age-related cognitive loss [1]. Ageing is also the biggest risk factor for developing dementia, an umbrella term encompassing disorders characterised by severe cognitive impairments in the elderly, such as Alzheimer's disease (AD) [2] [3] [4]. The unmet need for ameliorating age-related cognitive loss [5], as well as the lack of understanding as to what constitutes normal cognitive ageing can be addressed through well characterised cohorts, such as the Lothian Birth Cohort 1936 (LBC1936).

Participants of the LBC1936 originate from the Lothian region of Scotland, UK, and are part of a longitudinal study aiming to understand the aetiologies and mechanisms of people's differences in cognitive ageing [6]. These individuals were first tested at 11 years of age in 1947 using a general intelligence test and since the age of 70 have been cognitively re-evaluated every 3 years. In addition to this longitudinal data on cognition, the LBC1936 study has accumulated an extensive database on genetics, biomedical, social and lifestyle factors, and longitudinal brain imaging, resulting in a highly characterised cohort [6] [7] [8] [9]. Post-mortem brains have been donated by 9 non-demented individuals, and to date, there is pre-mortem authorisation for brain donation from 173 individuals. A pilot characterisation of the first brain donor demonstrated remarkable preservation of synaptic integrity in the LBC1936 participant compared to an Alzheimer's patient [10].

Neurodegenerative diseases are often characterised by the accumulation of misfolded

proteins. In Alzheimer's disease, amyloid- β (A β) forms extracellular protein aggregates, or A β plaques, and the synaptotoxic oligomeric form is now shown to be a key driver of dementia-associated cognitive impairments [11] [12] [13]. In addition, the microtubule stabilising protein tau can form neurofibrillary tangles (NFTs) when hyperphosphorylated. The combination of A β plaques and extensive NFTs are the hallmark of AD and are strongly implicated in cognitive decline and synapse degeneration [12]. These pathologies also often accumulate in healthy, non-demented individuals in an age-related fashion, but it is not yet clear whether they contribute to a mild cognitive decline in the absence of frank dementia [14]. Cognitive ageing and post-mortem pathology have been previously correlated in the Rush Religious Orders Study and the Rush Memory and Aging Project, where A β and tau were negatively associated with cognitive function [15] [16]. The LBC1936 neuropathology assessment aims to extend the characterisation from pre-mortem cognitive performance to post-mortem neuropathology in an attempt to discover underlying pathological changes that may explain the clinical phenotype. We have broadened the extent of post-mortem pathology investigated from well-established amyloid and tau analysis to the quantification of glial cell numbers. Specifically, microglia and astrocytes are immune cells essential for maintaining neuronal health through a range mechanisms, including synaptic pruning [17] [18], phagocytosis [19], and myelin regeneration [20]. During ageing, however, microglia and astrocytes become over-activated [21] [22] resulting in neuroinflammation and neurodegeneration [23] [24] [25]. The combination of these protein accumulations and cellular changes in the ageing brain likely contribute to the cortical thinning observed in the elderly, and the devastating atrophy observed in individuals with dementia [26].

For this study, we have used immunohistochemistry to study five brain regions from the nine LBC1936 brain donors. Four of the regions chosen are implicated in cognitive change during ageing and neurodegenerative diseases: Brodmann area (BA) 41/42 - superior temporal gyrus, BA44/45 - inferior frontal gyrus, BA46 - dorsolateral prefrontal cortex, and hippocampus. BA17, the primary visual cortex, was chosen as it is one of the cortical areas that is relatively spared during Alzheimer's disease [3] [27].

We found a regional variability in both pathological protein accumulation and gliosis in our nine brains. On average, the hippocampus appeared to have the highest level of NFTs and glial coverage, yet contained the lowest burden of amyloid. This study shows that regional variability in brain changes is likely a common feature in aged brains and that the LBC1936 cohort is an excellent group to study the changes associated with cognitive changes during ageing. Ultimately this will lead to a greater understanding of the cellular and molecular changes both in healthy ageing and in the early stages of neurodegeneration leading to dementia, such as mild cognitive impairment (MCI).

Objective

To quantify A β plaques, NFTs, gliosis, and cortical thickness in the nine post-mortem brains of nine LBC1936 non-demented aged individuals.

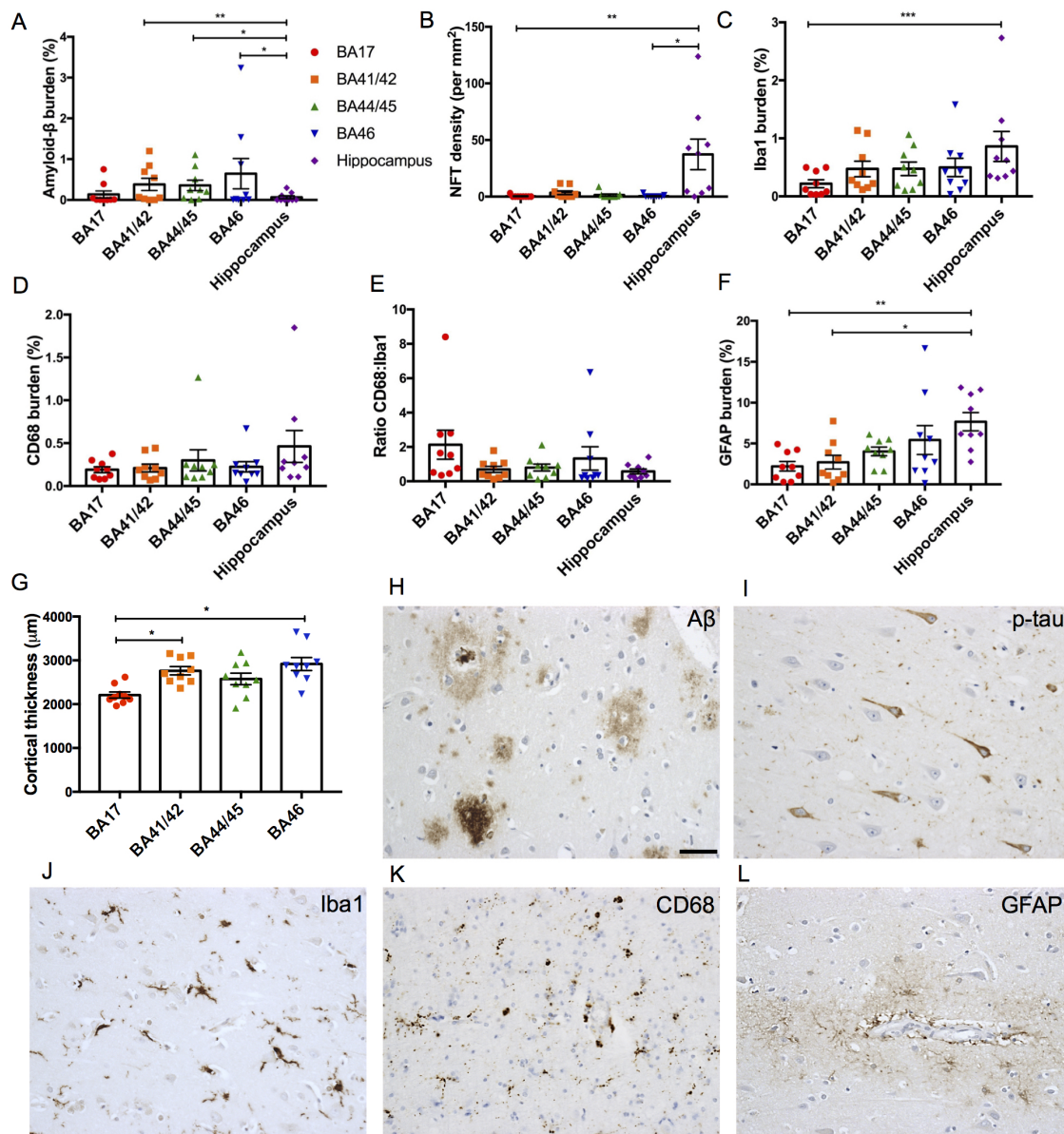


Figure Legend

Figure 1. Quantifying A β , NFTs, gliosis, and cortical thickness in LBC1936 participants' post-mortem brain tissue.

Measurements in each of the five brain regions and representative images of each stain: **A,H** amyloid- β , **B,I** phosphorylated tau/ neurofibrillary tau tangles (NFTs), **C,J** Iba1, **D,K** CD68, **E** ratio of CD68 to Iba1 microglia, **F,L** GFAP, and **G** cortical thickness.

Each data point represents one individual ($n=9$). Data are mean \pm standard error of the mean (SEM). For statistical analysis, Friedman test with Dunn's post-hoc, where * $p=0.05$, ** $p=0.01$, and *** $p=0.001$. Scale bar: 150 μ m.

Results & Discussion

Amyloid- β and Tau

A β burdens were significantly variable between the 5 brain regions from the 9 individuals (Friedman test, $p=0.0018$). Specifically, we found that the hippocampus had the lowest amount of A β plaques compared to BA41/42 ($p=0.0061$), BA44/45 ($p=0.0175$), and BA46 ($p=0.0365$) but not BA17 (Dunn's post-hoc test) (Figure 1A and H). The deposition

patterns between individuals varied considerably, with some showing very low A β burdens (SD017/16) and some showing extensive A β deposits throughout the grey matter (SD031/16). Strikingly, the participant with the highest A β burden (SD031/16) had an apolipoprotein (*APOE*) ϵ_4 allele, a strong late-onset AD risk factor, and a Thal score 5, resembling AD-like pathology (Supplementary tables 1 and 2). Moreover, cerebral amyloid angiopathy (CAA) with A β deposits around blood vessels was observed in some cases but was not always associated with A β plaques in the cortex (data not shown). Therefore, A β depositions are heterogeneous both between and within individuals.

As expected from the early Braak stages of the individuals, BA17 and BA46 showed almost no phosphorylated tau species, whereas BA41/42 and BA44/45 showed low NFT densities (Figure 1B and I). In contrast to our A β data, the hippocampus most commonly exhibited tau pathology, and significantly higher levels of tau pathology than BA17 ($p=0.0026$) and BA46 ($p=0.0224$) (Dunn's post-hoc test). The only individual (SD031/16) with tau spread in all five brain regions also had the highest NFT density across all regions. This was also the case with the highest amyloid burdens and an Apo ϵ_4 allele. Again, these preliminary data show evidence of p-tau heterogeneity not only between brain areas but also between individuals.

Microglia and astrocytes

Microglia and astrocytes, similar to tau, were found at highest levels in the hippocampus. Iba1-positive microglia, representing total microglia numbers, showed a significant difference in burdens between brain areas ($p=0.0017$), with the hippocampus having a higher burden than BA17 ($p=0.0006$) (Dunn's post-hoc test) (figure 1C and J). CD68-positive microglia, a marker of phagocytic activity, showed no significant differences between the five brain areas ($p=0.135$) (figure 1D and K). By exploiting the fact that Iba1 stains most microglia and CD68 only stains phagocytic microglia, we generated a ratio of phagocytic versus total microglia in all five regions. No statistical differences of activation status were observed between brains areas ($p=0.216$) (figure 1E).

Astrocyte burdens were significantly elevated in the hippocampus compared to both BA17 ($p=0.0061$) and BA41/42 ($p=0.0287$), but no further significant differences in burdens were found between other brain areas (Dunn's post-hoc test) (figure 1F and L). Altogether, the hippocampus has statistically higher levels of glial cells than other brain areas, particularly BA17.

Cortical thickness

A significant difference in cortical thickness between cortical regions was observed ($p=0.0133$), with BA17 showing a significantly thinner cortex than BA41/42 ($p=0.037$) and BA46 ($p=0.0209$), but not BA44/45 (Dunn's post-hoc test) (figure 1G). These data show BA17 has a thinner cortex compared to more anterior areas. This observation is most likely explained by natural rostro-caudal differences in cortical thickness, rather than ageing-induced neuron loss [28].

Discussion

In this study, we have measured A β , phosphorylated tau (NFT), microglia, and astrocyte levels in five brain regions and have demonstrated both regional and individual variability in nine non-demented LBC1936 participants.

Our observed heterogeneity in tau and A β burdens heterogeneity reflects previous findings in the literature. Specifically, AD brains have higher levels of tau pathology in the hippocampus compared to cortical regions [11] [29]. Conversely, A β deposits are highest in the neocortex and moderately distributed in the hippocampus, until later stages of the disease [11] [29]. The greatest genetic risk factor for developing AD in non-familial cases is the possession of an *APOE* ϵ_4 allele, whereas the ϵ_2 allele appears to be protective against AD [30]. Of note, the highest amyloid and tau pathology was observed in the only individual with an ϵ_4 allele and the lowest amyloid pathology in the only individual with the ϵ_2 allele (supplementary table 1). Nevertheless, these results have to be interpreted with caution due to the extremely low numbers involved and that the levels of A β and NFTs in this cohort are significantly lower than those found in AD cases. Overall, despite the small sample size, the data from this study have surprisingly, yet closely, depicted previously described features of the ageing brain.

It is currently unclear if or how A β and tau interact in the ageing and AD brain. It is more evident that the quantity and spread of NFTs correlates strongly with, not only, cognitive

decline but also synapse loss and gliosis [31] [32]. Indeed, in our study, glial cells were predominantly found in the hippocampus in the LBC1936 individuals (similar to NFTs), confirming preceding findings [33] [34]. The increased glial coverage and p-tau in the hippocampus may indicate a causative role in the early hippocampal neurodegeneration during ageing and AD, and by extension it may explain the early memory impairments observed in the elderly and demented. Interestingly, other studies have shown that Iba1+ve microglia burdens are correlated with normal cognitive function, whereas activated (CD68) microglia are correlated with poorer cognitive function in dementia [35]. While this study is currently too small to assess these kinds of associations, as more brains become available this cohort will provide an invaluable opportunity to discover associations between post-mortem brain changes and detailed longitudinal cognitive performance.

The hippocampus undergoes extensive spine remodelling [36] [37] and as a result, may require greater glial surveillance to ensure efficient temporal and spatial synaptic pruning. This could explain the higher numbers of glial cells we observe in the hippocampus compared to cortical regions (figure 1C). Synaptic health is critical for normal brain function, evidenced by the fact that synapse loss is the best correlate with cognitive impairment in AD [38] [39]. Synapse loss is also thought to correlate with poorer cognitive performance in normal ageing [40]. During ageing, synaptic degeneration has been shown to impair electrophysiological properties of neurons by increasing the long-term depression (LTD) and reducing the long-term potentiation (LTP) [41] [42]. Notably, A β and p-tau co-localize in synapses in AD brains [29], while microglia and astrocytes interact with synapses both in health and disease, marking synapses as critical points in normal and pathological ageing [43]. Altogether, these age-related synaptic changes are hypothesised to occur before the onset of cognitive loss and to be key drivers of MCI and dementia [44]. It would, therefore, be important to quantify synaptic puncta in the LBC1936 participants using high-resolution techniques such as array tomography to visualize how microglia, astrocytes, A β , and NFT's interact with synapses. Furthermore, the data presented here must be compared to a younger cohort to establish if these brain area differences truly are age-associated phenomena, or region-specific throughout life. In the future, a greater sample size will allow us to correlate longitudinal cognitive function scores to synapse integrity and tau pathology or gliosis, in order to understand how these factors may mediate age-related cognitive impairments. Furthermore, by following-up on these individuals' cognitive function, we expect to detect MCI in some individuals and through our detailed post-mortem analyses, begin to get an understanding of its neuropathological origin. By doing so, prodromal phases of AD can be detected early and described post-mortem to the single synapse level, which will greatly improve our understanding of AD progression and thus provide better avenues for treatments.

Conclusions

To summarise, A β plaques, NFTs, microglia, and astrocytes were differentially distributed in the brains of the nine LBC1936 post-mortem cases, with NFTs and glial cells, but not A β , being elevated in the hippocampus. These data extend the phenotyping of this well-characterised cohort and form a building block for future studies of the neurobiological substrates of cognitive ageing.

Limitations

The greatest limitation of this study is the small sample size ($n=9$). At the moment, the sample size is not large enough to make meaningful conclusions about the cellular and protein differences between brain areas, nor provide a causative relationship between the variables, and age-related impairments. However, as the post-mortem tissue availability increases, the study will be powered enough for making more robust conclusions.

Additional Information

Methods and Supplementary Material

Please see <https://sciencematters.io/articles/201708000003>.

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Ethics Statement

Use of human tissue for post-mortem studies has been reviewed and approved by the Edinburgh Brain Bank ethics committee and the ACCORD medical research ethics committee, AMREC (approval number 15-HV-016; ACCORD is the Academic and Clinical Central Office for Research and Development, a joint office of the University of Edinburgh and NHS Lothian). The Edinburgh Brain Bank is a Medical Research Council funded facility with research ethics committee (REC) approval (11/ES/0022). Tissue from nine donors was used for this study and their details are found in supplementary information.

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